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Autoregulation of Nisin Biosynthesis in *Lactococcus lactis* by Signal Transduction*

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The post-translationally modified, antimicrobial peptide nisin is secreted by strains of *Lactococcus lactis* that contain the chromosomally located nisin biosynthetic gene cluster *nisABTCIPRKFEG*. When a 4-base pair deletion is introduced into the structural *nisA* gene (Δ*nisA*), transcription of Δ*nisA* is abolished. Transcription of the Δ*nisA* gene is restored by adding subinhibitory amounts of nisin, nisin mutants, or nisin analogs to the culture medium, but not by the unmodified precursor peptide or by several other antimicrobial peptides. Upon disruption of the *nisK* gene, which encodes a putative sensor protein that belongs to the class of two-component regulators, transcription of Δ*nisA* was no longer inducible by nisin. Fusion of a *nisA* promoter fragment to the promoterless reporter gene *gusA* resulted in expression of *gusA* in *L. lactis* NZ9800 (Δ*nisA*) only upon induction with nisin species. The expression level of *gusA* was directly related to the amount of inducer that was added extracellularly. These results provide insight into a new mechanism of autoregulation through signal transduction in prokaryotes and demonstrate that antimicrobial peptides can exert a second function as signaling molecules.

Nisin is an antimicrobial peptide (1–3) widely used in the food industry as a safe and natural preservative. The ribosomally synthesized precursor peptide undergoes extensive post-translational modification, which includes dehydration of serine and threonine residues and the formation of thioether bridges called (β-methyl)lanthionines, resulting in five ring structures named A, B, C, D, and E (Fig. 1*B*). Peptides containing these characteristic modified residues are named lantibiotics (4). Eleven genes organized in a cluster have been implicated to be involved in the complex biosynthesis of nisin, *i.e. nisABTCIPRKFEG* (Fig. 1*A*) (5–11). Of these genes, *nisA* encodes the nisin A precursor peptide of 57 amino acid residues; *nisB* and *nisC* encode putative enzymes involved in the post-translational modification reactions (based on homology to genes found exclusively in other lantibiotic gene clusters); *nisT* encodes a putative transport protein of the ABC translocator family that is probably involved in the extrusion of modified precursor nisin (7, 9); *nisP* encodes an extracellular subtilisin-like protease involved in precursor processing (8); *nisI* encodes a lipoprotein involved in the producer self-protection against nisin (9); and *nisFEG* encodes putative transporter proteins that have also been implied in immunity (11). A schematic representation of the post-translational events yielding mature nisin A is shown in Fig. 1*B*. Nisin Z is a natural variant of nisin A that contains an asparagine residue at position 27 instead of the histidine residue found in nisin A (12). Both nisin A- and nisin Z-producing strains are common in nature, and both structural genes (*nisA* and *nisZ*) have been cloned (5, 6, 13).

The proteins encoded by *nisR* (8) and *nisK* (10) have shown to be involved in the regulation of nisin biosynthesis (8, 10). NisR is a response regulator, and NisK is a sensor histidine kinase which belong to the class of two-component regulatory systems (14–16). When the genes *nisABTCIR* are present on a multicopy plasmid, production of fully modified precursor nisin is observed, indicating that overexpression of *nisR* alone is sufficient to activate transcription of *nisA* and obviously also of the biosynthetic genes downstream by partially reading through an inverted repeat sequence (Fig. 1*A*) (8). This observation is similar to the regulation of expression of *iep* and *degU* genes in *Bacillus subtilis*, where overexpression of the response regulator activates transcription of the target genes (17), and to the case of overexpression of *epiQ*, which encodes a response regulator involved in the biosynthesis of the lantibiotic epidermin (18). When only the genes *nisABTCI* are present on a multicopy plasmid (pNZ9000) in *Lactococcus lactis* MG1614, no transcription of *nisA* is observed (9). Two gene products have been identified for the regulation of the biosynthesis of the related lantibiotic subtilin (19), which also belong to the class of two-component regulators, *i.e.* SpaR, the response regulator, and SpaK, the sensor histidine kinase (20, 21). Upon disruption of either of these genes, subtilin production was abolished, indicating the involvement of these gene products in subtilin biosynthesis (20). The regulation was shown to be growth-phase dependent, but an inducing signal was not identified (20, 21).

While the structure and function of two-component regulators have been studied in great detail (14–16), the nature of the inducing signal has remained unclear in many cases. It is demonstrated here that fully modified nisin can induce the transcription of its own structural gene as well as of the downstream genes by limited read-through, via signal transduction, by acting as the extracellular signal for the sensor histidine kinase NisK.

MATERIALS AND METHODS

Strains and Plasmids—L. lactis strains MG1614 (22), NZ9700 (a nisin-producing transconjugant containing Tn*5276*) (23), and NZ9800

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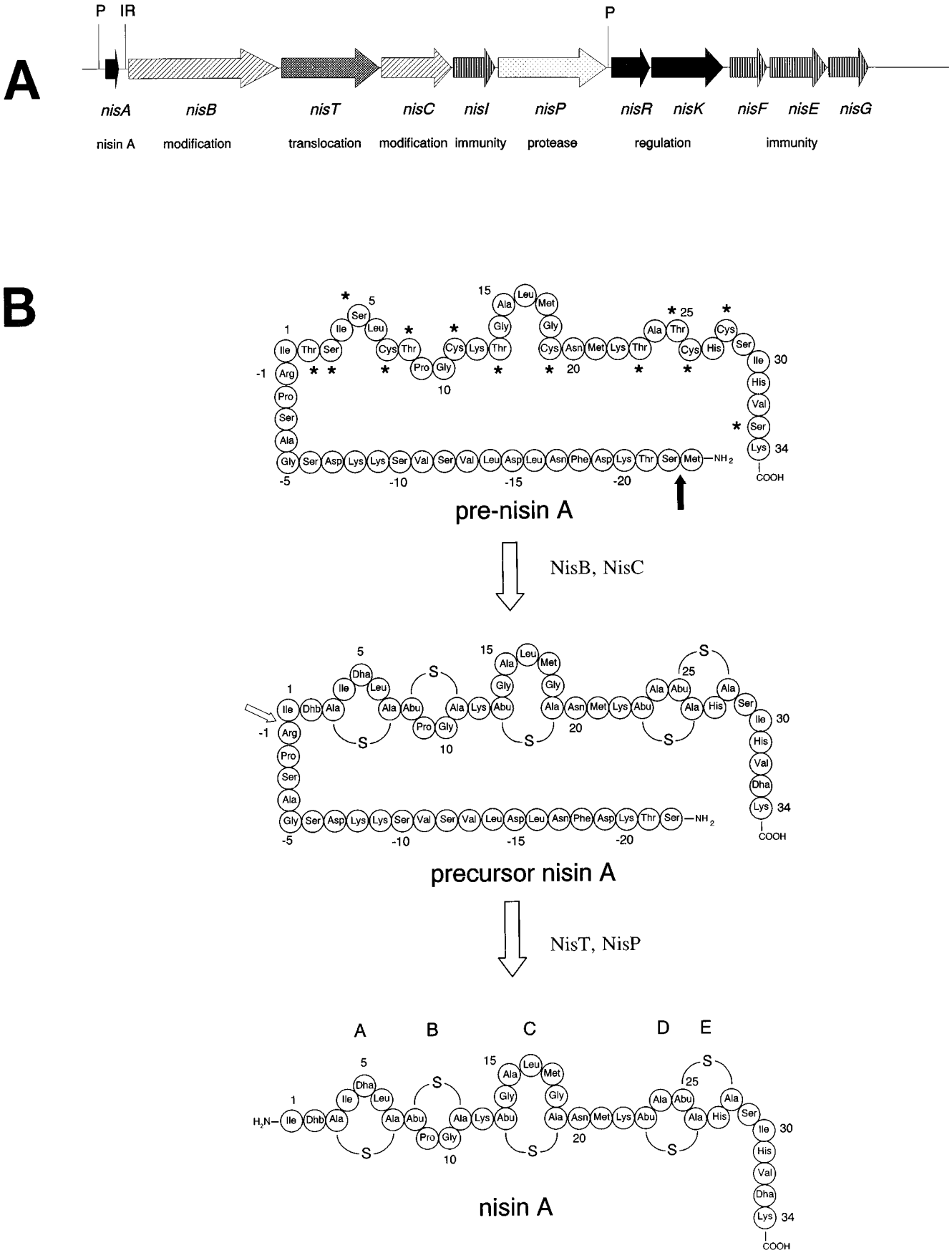

FIG. 1. *A*, organization of the nisin gene cluster. Established (*nisAIPRKFEG*) and putative (*nisBCT*) functions of the gene products have been indicated. *P* denotes a mapped promoter, and *IR* denotes an extensive inverted repeat sequence that could act as a rho-independent terminator (7). *B*, schematic outline of the biosynthesis of nisin A. Rings are labeled *A–E*. *Asterisks* indicate residues that will be modified. The *black arrow* indicates processing of the N-terminal Met residue, while the *small white arrow* indicates processing of the leader peptide by the action of NisP (8). *Dha*, dehydroalanine; *Dhb*, dehydrobutyrine.

(a derivative of NZ9700 in which the *nisA* gene has been exchanged by replacement recombination with a modified *nisA* gene containing a 4-bp [1] deletion in the pronisin-encoding part (Δ*nisA*) and which is therefore no longer able to produce nisin A) have been described previously (9). *L. lactis* strains were cultivated without aeration at 30 °C in M17 broth (Difco) supplemented with 0.5% (w/v) glucose (GM17) or sucrose (SM17). For *L. lactis* strains harboring pNZ273-derived plasmids (24), media were supplemented with 10 μg/ml chloramphenicol. Expression plasmids pNZ9010 and pNZ9013 (9, 25), containing the *nisA* and *nisZ* genes, respectively, under control of the efficient *lac* promoter, were introduced into *L. lactis* strain NZ9800, leading to the production of nisin A or nisin Z in similar amounts as in *L. lactis* wild-type strain NZ9700 (25). As a host strain for cloning experiments, *Escherichia coli* strain MC1061 (26) was used.

The *nisA* promoter region including part of the *nisA* gene was isolated as a 1442-bp *Bgl*II-*Ecl*136II fragment from plasmid pNZ9000 (8). This fragment was cloned into pNZ273, containing the promoterless *gusA* gene (24), which had been digested with *Bgl*II and *Sca*I, generating plasmid pNZ8003. Part of the upstream promoter region was deleted by digesting pNZ8003 with *Bgl*II and *Tth*111I. These sites were made blunt by Klenow polymerase and ligated, generating plasmid pNZ8008, which eventually contained a 312-bp *nisA* promoter fragment in front of the *gusA* gene. Another part of the *nisA* promoter region, including the full *nisA* gene and the first part of the *nisB* gene, was isolated as a 1904-bp *Bgl*II-*Mun*I fragment from plasmid pNZ9000. This fragment was cloned into pNZ273 (24), which had been digested with *Bgl*II and *Eco*RI, generating plasmid pNZ8002. A 1442-bp *Bgl*II-*Ecl*136II promoter fragment was deleted in pNZ8002, generating pNZΔ8002, by making the *Bgl*II site blunt with Klenow polymerase and subsequent ligation to the *Ecl*136II site. All constructs were initially made in *E. coli* MC1061 (26). Plasmids pNZ8008, pNZ8002, and pNZΔ8002 were used to transform *L. lactis* NZ9700 and *L. lactis* NZ9800 (9), and transformants were obtained by selecting for resistance to chloramphenicol.

DNA Techniques and DNA Sequence Analysis—Restriction enzymes and other DNA-modifying enzymes were purchased from Life Technologies, Inc. or U. S. Biochemical Corp. and used as recommended by the manufacturers. The DNA sequence of the *nisZ* region was determined on the double-stranded plasmid DNA with the *Hin*dIII primer by the chain termination method (27). Transcription analyses of the *nisA* and Δ*nisA* genes were performed by isolation of RNA from *L. lactis* strains NZ9700 and NZ9800, Northern blotting, and subsequent hybridization with a radiolabeled *nisA* probe as described previously (9). RNA isolation was performed 2 h after induction of a culture with an $A_{600\ nm}$ of 0.5. RNA (20 μg) was loaded in each lane, and the amounts were estimated by comparing the intensity of the 16 S and 23 S RNA bands.

Inactivation of Chromosomal nisK and nisB by Gene Replacement—The chromosomal copy of the *nisK* gene was inactivated by introduction of an erythromycin resistance gene (28) into the open reading frame of *nisK*. For this purpose, a 2.8-kilobase pair *Hin*dIII-*Eco*RI chromosomal DNA fragment from strain NZ9700 containing the 3′-part of the *nisP* gene and the intact *nisR* and *nisK* genes was cloned into pUC19. The erythromycin resistance gene was introduced into a unique *Sal*I site, resulting in the interruption of the *nisK* open reading frame between the encoded amino acids 9 and 10 of NisK and leaving 1.1 and 1.7 kilobase pairs of flanking regions at the 5′- and 3′-ends, respectively. This construct was designated pNZ9150. Strain NZ9800 was transformed with the nonreplicating plasmid pNZ9150, and integrants were selected on plates containing erythromycin (2.5 μg/ml). The selected integrants were analyzed by polymerase chain reaction using primers 5′-CGGTCAATCTCGGAG-3′ and 5′-CGCTTTGTAATCATTTTCATC-3′ and by Southern hybridization using pUC19 DNA (29) as a probe. In one of the strains (NZ9850), the erythromycin resistance gene had been integrated via gene replacement at the 3′- and 5′-flanking regions, introducing this gene into the open reading frame of the chromosomal copy of the *nisK* gene, in the absence of any pUC19 sequences. The resulting construct was further analyzed by polymerase chain reaction of the *nisK* region and by Southern blotting using *nisK* as a probe to confirm the desired configuration.

The *nisB* gene was disrupted by introducing a 162-bp in-frame deletion into the middle of the gene. This was accomplished by cloning a 4.4-kilobase pair *Bgl*II-*Eco*RI fragment, containing *nisB* and surrounding regions from the nisin gene cluster, into a *Bam*HI-*Eco*RI-digested pUC19 vector, which harbored an additional erythromycin resistance marker, as has been described previously (9). The deletion was made by removing an internal *Hpa*I fragment from the *nisB* gene and subsequent ligation. The resulting plasmid was named pNZ9135 and was used for transformation of *L. lactis* NZ9700. Following transformation, erythromycin-resistant colonies were obtained that had integrated the plasmid by recombination of the plasmid with one of the flanking regions of the deleted fragment. After growing for 200 generations in the absence of erythromycin and plating, a colony was obtained that was sensitive to erythromycin. This had apparently been caused by a second recombination event involving the flanking region on the other side of the deletion than the side of the first recombination event, resulting in the replacement of *nisB* with Δ*nisB* on the chromosome. The configuration of the desired construct was confirmed by polymerase chain reaction analysis of the *nisB* region with the deletion and by Southern analysis of *Bgl*II-digested chromosomal DNA. The desired strain was called NZ9700 Δ*nisB*.

Production, Purification, and Characterization of Nisin Mutants—Mutants of nisin Z were produced as described previously (25). The primers used for site-directed mutagenesis of the *nisZ* gene were as follows: 5′-CAGGTGCATCACCACGC**TGG**ACAAGTATTTCGCTATGTAC-3′ (I1W), 5′-CACCACGCATT**T**CAAGTATTTCGCTATG-3′ (T2S), 5′-CACCACGCATTACAA**CA**ATTTCGCTATGTACACCC-3′ (S3T),and 5′-AACAGGAGCTCTG**TG**GGGTTGTAACATG-3′ (M17W) (mutated nucleotides are indicated in boldface). All mutants were purified to homogeneity, and the structures of the modified residues were confirmed by one- and two-dimensional ^{1}H NMR (25). It was established that T2S nisin Z contains a dehydroalanine residue at position 2, S3T nisin Z has a β-methyllanthionine ring between residues 3 and 7 instead of a lanthionine, and I1W nisin Z and M17W nisin Z contain a Trp residue at positions 1 and 17, respectively. Precursor nisin Z, containing the subtilin leader peptide (sl-nisin Z), was obtained as described before (30). Purified lacticin 481 was isolated as described previously (31, 32). Purified Pep5 (33) was obtained from Dr. H.-G. Sahl (Bonn, Germany). Unmodified precursor nisin A was obtained from the laboratory of Dr. G. Jung (Tübingen, Germany). Preparations of subtilin (19) and lactococcin A (34) consisted of culture supernatants of producing strains, which were confirmed to possess substantial antimicrobial activity in agar diffusion assays. Antimicrobial activities against *L. lactis* MG1614 were determined essentially as described before for *Micrococcus flavus* (25). *L. lactis* was cultured in GM17 broth at 30 °C with an initial $A_{600\ nm}$ of 0.025, and outgrowth was measured when the culture without nisin had reached an $A_{600\ nm}$ of 0.8.

β-Glucuronidase Assays—Lactococcal cells (1 ml) were harvested at 1.5 h after induction with nisin (or mutants or fragments or other antimicrobial species) and adjusted in NaP_i buffer (50 mM $NaHPO_4$, pH 7.0) to a final $A_{600\ nm}$ of 2.0. The cells were permeabilized by adding 50 μl of acetone/toluene (9:1) per ml of cells followed by 10 min of incubation at 37 °C. The extracts were used immediately in the assay. For the determination of β-glucuronidase activity, 40 μl of extract was added to 950 μl of buffer (50 mM $NaHPO_4$, pH 7.0, 10 mM β-mercaptoethanol, 1 mM EDTA, 0.1% Triton X-100) and 10 μl of 100 mM *p*-nitro-β-D-glucuronic acid (CLONTECH, Palo Alto, CA). The mixture was incubated, and the increase in $A_{405\ nm}$ was measured at 37 °C. Histochemical screening for *gusA* positive colonies was performed by including 5-bromo-4-chloro-3-indolyl glucuronide (Research Organics Inc., Cleveland, OH) at a final concentration of 0.5 mM in GM17 plates (24). Purified nisin fragments (35–39) were obtained from Prof. T. Shiba (Protein Research Foundation, Osaka, Japan).

RESULTS AND DISCUSSION

*Transcription Analyses of Δ*nisA *in the Presence and Absence of Nisin or Nisin Mutants*—The promoter sequence and the transcription start site of *nisA* have been identified, and a transcript of ~260 nucleotides has been demonstrated in *L. lactis* strain NZ9700, which contains Tn*5276* (9). It has also been found that transcription of *nisA* is dependent on the integrity of *nisA* itself since a 4-bp deletion in the middle of the *nisA* gene (Δ*nisA*) on the chromosome of *L. lactis* strain NZ9800 completely abolishes transcription of this gene (9). For further transcription analyses of the structural and biosynthetic genes, a series of isogenic lactococcal strains was used, including the nisin-producing NZ9700 and non-nisin-producing NZ9800 strains.

Northern blotting showed that in strain NZ9800, the transcript of Δ*nisA* was absent, but after adding small amounts of

[1] The abbreviation used is: bp, base pair(s).

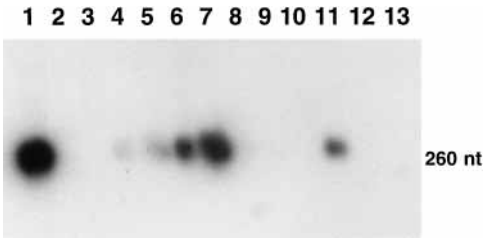

FIG. 2. **Northern blot prepared using *nisA* as a probe of RNA from several uninduced lactococcal cultures and cultures induced with different amounts of nisin A or unmodified precursor nisin A or with the lantibiotic Pep5.** *Lane 1*, NZ9700 (nisin A producer); *lane 2*, MG1614; *lanes 3–7*, NZ9800 with nisin A (0, 1, 2.5, 10, and 50 ng/ml, respectively); *lanes 8* and *9*, NZ9800 Δ*nisK* with nisin A (0 and 2.5 ng/ml, respectively); *lanes 10* and *11*, NZ9700 Δ*nisB* with nisin A (0 and 2.5 ng/ml, respectively); *lane 12*, NZ9800 with unmodified precursor nisin A (1000 ng/ml); *lane 13*, NZ9800 with Pep5 (1000 ng/ml).

nisin A to the culture medium at an $A_{600\,nm}$ of 0.5, *nisA* transcripts appeared again (Fig. 2). Interestingly, the amount of these transcripts was dependent on the amount of nisin A added (Fig. 2, *lanes 3–7*). Several other related peptides were able to induce transcription, such as nisin Z and various nisin Z mutants, *i.e.* T2S nisin Z, S5T nisin Z (25), M17W nisin Z, S3T nisin Z, and sl-nisin Z, a fully modified nisin Z species that has the subtilin leader peptide still attached (30). However, the last two species were >100-fold less effective inducers compared with nisin Z (data not shown). In contrast, the T2S and M17W nisin Z mutants were more potent inducers than nisin Z. These findings demonstrate that the modified lantibiotic part plays an important role in the induction process. Interestingly, several less related peptides evoked no restoration of transcription, *i.e.* the unmodified synthetic nisin A precursor of 57 amino acid residues (Figs. 1*B* and 2, *lane 12*), the 56% homologous lantibiotic subtilin (19), the lantibiotic lacticin 481 (31, 32), the lantibiotic Pep5 (Fig. 2, *lane 13*) (33), and the antimicrobial peptide lactococcin A (34) (data not shown for subtilin, lacticin 481, and lactococcin A).

Determination of the Induction Capacity of Nisin (Mutants) by Use of the Nisin Promoter Fragment Fused to the Reporter Gene gusA—To obtain a more quantitative assay of induction capacity and to investigate whether the *nisA* promoter could be used to regulate expression of heterologous genes in *L. lactis*, a nisin promoter fragment of 312 bp containing part of the *nisA* structural gene was fused to the promoterless reporter gene *gusA* of *E. coli* on plasmid pNZ273 (24). This construct, named pNZ8008, was used to transform strain NZ9800. The resulting strain was assayed for β-glucuronidase activity with and without induction by (mutant) nisins or other antimicrobial peptides. Without induction, β-glucuronidase activity could not be demonstrated, whereas wild-type nisin A and nisin Z effectively induced β-glucuronidase activity (Fig. 3). Moreover, the T2S and M17W nisin Z mutants were found to induce higher expression of *gusA* compared with wild-type nisin A and nisin Z, whereas the S3T and I1W nisin Z mutants were found to have lower induction capacity (Fig. 3). It was calculated that <5 molecules of the best inducer (T2S nisin Z) per cell are sufficient to activate transcription, which illustrates the high efficiency characteristic of signal transduction processes. Concordant with the transcription analyses, some of the antimicrobial peptides tested did not elicit induction of *gusA* expression (*i.e.* the unmodified synthetic nisin A precursor peptide, subtilin, lacticin 481, and lactococcin A). There is no direct relationship between antimicrobial activity of the nisin mutants against *L. lactis* strain MG1614 and their induction capacity (Table I). The difference in potency can be attributed to the observation that antimicrobial activity is dependent on pore-forming activity in membranes (40–42), while induction capacity is likely to be dependent on interaction (directly or indirectly) with NisK.

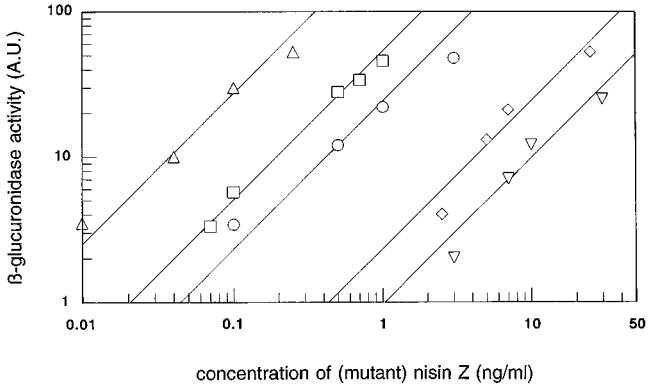

FIG. 3. **Dose response of purified (mutant) nisins as inducers of *gusA* expression in *L. lactis* strain NZ9800 harboring pNZ8008.** Nisin species were as follows: △, T2S nisin Z; □, M17W nisin Z; ○, wild-type nisin Z; ◇, S3T nisin Z; ▽, I1W nisin Z. Standard errors were <20% for each given value. *A.U.*, arbitrary units.

TABLE I
Comparison of induction capacity with antimicrobial activity against L. lactis strain MG1614 or against M. flavus of nisin Z, nisin Z mutants, and synthetic nisin A fragments (36)

Nisin Z (mutant)	Induction capacity[a]	Activity against *L. lactis*[b]	Rings present
	%	%	
T2S nisin Z	1100	78	All
M17W nisin Z	220	12	All
Nisin Z	100	100	All
S3T nisin Z	11	2	All
I1W nisin Z	3	47	All
Nisin A fragments (residues)	**Induction capacity**	**Activity against *M. flavus*[c]**	**Rings present**
	%	%	
Nisin A	100	100	A–E
1–21	30	<50	A–C
1–19	8	<25	A–C
1–11	2	<1	A, B
3–19 (L-Ala-5)	1	<1	A–C
3–19 (D-Ala-5)	ND[d]	<1	A–C
3–19	ND[d]	<1	A–C
8–19	ND[d]	<1	B, C
22–34	ND[d]	<1	D, E

[a] The induction capacity of nisin Z was taken as 100%; values were calculated by measuring the distances between the dose-response curves of nisin Z and each of the nisin species.
[b] The minimal inhibitory concentration of nisin Z against *L. lactis* MG1614 (14 ng/ml) or against *M. flavus* (11 ng/ml) was taken as 100% activity. All nisin fragments contained the modified residues as they are present in wild-type nisin A (Fig. 1*B*), unless indicated otherwise, at position 5. Standard errors were <20% for each given value.
[c] Values are taken from Ref. 36.
[d] ND, not detectable.

In further experiments, the nisin-producing strain NZ9700 with either plasmid pNZ273 (containing the promoterless *gusA* gene) or pNZ8008 (containing the *nisA* promoter fragment followed by the *gusA* gene) was used in an agar diffusion assay (8) to determine the amount of nisin produced. Fifty times lower nisin production and severely reduced immunity were observed when plasmid pNZ8008 was present compared with the situation where pNZ273 was present. This can be explained by titration of the response regulator NisR by the multicopy presence of the *nisA* promoter region containing the putative NisR-binding site.

Structural Requirements of the Inducer Molecule Tested by Use of Synthetic Nisin Fragments—More detailed insight into

the minimal structural requirements of the inducer molecule was obtained by using synthetic nisin A fragments (35–39) in the *gusA* reporter assay (Table I). The minimal requirement for retaining induction capacity (2% induction of that of nisin A) was the presence of residues 1–11 of nisin A, comprising the first two rings. Addition of the third ring enhanced induction (8–30% induction), whereas a severe decrease in induction was caused by deleting the N-terminal residues Ile-1 and dehydrobutyrine 2 (0–1% induction) (Table I). Fragments that contained rings B and C or rings D and E (for nomenclature of rings, see Fig. 1*B*) were not capable of acting as a signal effector. Thus, the most probable site of molecular interaction with the sensor protein NisK will be residues 1–11 of the nisin molecule.

Requirement of nisK Expression for Signal Transduction—The sequence of the *nisK* gene located on Tn*5276* has been reported (43) and was found to be identical to that of *nisK* from *L. lactis* 6F3 (10). The chromosomal *nisK* gene was insertionally inactivated by introduction of an erythromycin resistance gene (28) into strain NZ9800, yielding strain NZ9850. As expected, transcription of Δ*nisA* was no longer inducible by any of the nisin species (Fig. 2, *lanes 8* and *9*). Nisin production in strain NZ9850 could not be restored by introduction of plasmid pNZ9010 (*nisA*) or pNZ9013 (*nisZ*), whereas it could be restored in strain NZ9800. Since the immunity level of strain NZ9850 is similar to that of strain MG1614 (0.01 μg of nisin A/ml), induction experiments were performed with amounts of nisin well below this level. Under these conditions, normal growth of the cells was observed. Strain NZ9850 was also transformed with pNZ8008, but after induction with 0.0005–0.0025 μg of nisin A/ml, no β-glucuronidase activity could be measured (<0.3 arbitrary unit), indicating at least 200 times lower expression than in strain NZ9800 containing pNZ8008, with the same inducer concentrations (Fig. 3). No polar effects of the *nisK* disruption on expression of the *nisFEG* genes downstream of *nisK* are expected since a promoter has been indicated in front of *nisFEG* (11).[2] Moreover, the *nisR* and *nisK* genes have been integrated on the chromosome of strain MG1614 by replacement recombination, and the resulting strain was transformed by pNZ8008. In this strain, *gusA* expression was inducible by nisin species (data not shown), proving that only *nisR* and *nisK* are required for signal transduction. These results clearly demonstrate that NisK is essential in the signal transduction pathway and probably interacts directly with nisin itself.

Effects of Disruption of nisB on Transcription of nisA and Downstream Genes—An in-frame deletion in the *nisB* gene of *L. lactis* strain NZ9700, made by replacement recombination, abolished nisin production as well as transcription of *nisA*, demonstrating that a hampered biosynthesis of nisin abolishes transcription of *nisA*. In this case, transcription of *nisA* could be restored by addition of nisin to the cells (Fig. 2, *lane 11*), probably because of the presence of intact *nisR* and *nisK* genes, which have their own promoter. The transcription start site of *nisRK* was mapped by primer extension and shown to be an A nucleotide 26 nucleotides upstream of the start codon of *nisR* (position 2117 in the nucleotide sequence published in Ref. 8). To probe the influence of a large inverted repeat sequence located in the intergenic region between Δ*nisA* and *nisB* on expression of genes downstream of Δ*nisA* (Fig. 1*A*), another plasmid was constructed (pNZ8002) in which the nisin promoter fragment including Δ*nisA* as well as the intergenic region and the first part of *nisB* was fused to the *gusA* gene. This

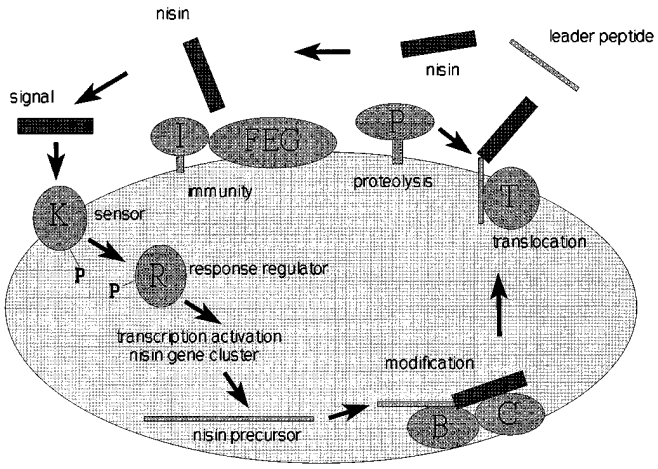

FIG. 4. **Model for nisin biosynthesis and regulation.** In Step 1, NisK senses the presence of nisin in the medium and autophosphorylates. In Step 2, the phosphate group is transferred to NisR, which acts as a transcriptional activator, followed by mRNA synthesis and ribosomal synthesis of unmodified precursor nisin and of biosynthetic proteins. In Step 3, the precursor is modified by the putative enzymes NisB and NisC (7, 9). In Step 4, the fully modified precursor peptide is translocated across the membrane by the putative ABC transporter NisT (7, 9). In Step 5, fully modified precursor nisin is extracellularly processed by NisP (8), resulting in the release of active nisin. NisI (9), together with NisF, NisE, and NisG (11), protects the cell from the bacteriocidal action of nisin by a thus far unknown mechanism.

plasmid was able to direct expression of *gusA* in strain NZ9800 only after induction with nisin species, albeit to an ~50-fold reduced level relative to *gusA* expression in pNZ8008 in strain NZ9800. When the nisin promoter fragment was removed from pNZ8002, yielding pNZΔ8002, β-glucuronidase activity was completely abolished, even in the presence of an inducer. These results show that expression of at least one downstream gene, *i.e. nisB*, is coregulated and is dependent on the presence of the *nisA* promoter. Most likely, expression of the other downstream genes *nisTCIP* limited read-through is also dependent on the *nisA* promoter since a significant increase in immunity levels, for which NisI is partially responsible (9), was found in the induced state relative to the uninduced state of strain NZ9800. Moreover, no apparent promoter sequences were found in front of any of the genes *nisBTCIP*.

Conclusion—We have demonstrated that transcription of *nisA* is autoregulated, not intracellularly by its direct translation product, but extracellularly by the secreted and fully modified peptide via signal transduction by a two-component regulatory system. A model based on previous work (5–11) and on this study shows the possible sequence of events with regard to nisin biosynthesis and regulation (Fig. 4).

Mutants of nisin or precursors of nisin that have the leader peptide attached to the mature lantibiotic (second molecule shown in Fig. 1*B*) can also act as inducers, whereas other antimicrobial peptides are incapable of induction. The presence of the modified residues is of crucial importance for induction capacity, especially those present in the N-terminal part of nisin. To our knowledge, this is the only report of peptides that can induce transcription of their own structural gene via signal transduction. Interestingly, a recent report on syndecan biosynthesis in mice, which plays a role in wound repair, describes the role of the antimicrobial peptide PR39 in induction of syndecan gene transcription (44), although the amount of inducer needed (0.5 mM) is at least a factor of 10,000 higher than for nisin (30 pM). This suggests that the role of antimicrobial peptides in nature might be broader than just the antagonistic action because in some cases these peptides can also act as signals for transcription activation of their own structural gene

[2] P. G. G. A. de Ruyter, O. P. Kuipers, and W. M. de Vos, unpublished data.

or of other genes. There may be several evolutionary reasons for the autoregulation of nisin gene transcription via signal transduction, *e.g.* (i) to save energy by control of the integrity of the gene cluster since any dysfunctional biosynthetic gene will abolish inducer formation and thus expression of biosynthetic genes; (ii) to raise immunity levels in response to high nisin production by neighboring cells, in other words, to amplify the response to environmental signals; or (iii) to promote cell to cell communication that allows the production of antimicrobial peptides in high quantities in a concerted action, thereby decreasing the chance of resistance development in target organisms.

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